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Insights into the initiation of type 2 immune responses

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Summary

Type 2 immune responses, characterized by the differentiation of CD4$^+$ T helper type 2 (Th2) cells and the production of the type 2 cytokines interleukin-4 (IL-4), IL-5, IL-9 and IL-13, are associated with parasitic helminth infections and inflammatory conditions such as asthma and allergies. Until recently the initiating factors associated with type 2 responses had been poorly understood. This review addresses the recent advances in identifying the diverse range of antigens/allergens associated with type 2 responses and the function, expression and sources of type-2-initiating cytokines (thymic stromal lymphopoietin, IL-25 and IL-33). We also discuss the latest findings regarding innate lymphoid cells, such as nuocytes, as early sources of type 2 cytokines and their importance in protective immunity to helminth infections. These developments represent major breakthroughs in our understanding of type 2 immunity, and highlight the increased complexity existing between the innate and adaptive arms of these responses. These additional steps in the type 2 immune pathway also offer potential targets for therapeutic intervention.

Keywords: allergens; interleukin-25; interleukin-33; innate lymphoid cells; nuocytes; T helper type 2 cells; thymic stromal lymphopoietin; type 2 responses

Introduction to T helper type 2 immune responses

The host immune system comprises both non-specific innate immunity and antigen-specific adaptive immune responses. Although described as separate entities there is extensive cross-talk between the innate and adaptive immune responses that together are important for combating the diverse array of microorganisms encountered by the host throughout its life.

Activated type 2 immune responses are typically characterized by the expression of classical effector type 2 cytokines including interleukin-4 (IL-4), IL-5, IL-9 and IL-13 that in turn affect antibody class switching to IgG1 and IgE, recruitment of inflammatory effector cells such as eosinophils, basophils and mast cells, and goblet cell hyperplasia leading to mucus production (Fig. 1). Ultimately, these effector functions have evolved to control extracellular helminth infections such as those involving *Schistosoma mansoni* and *Trichuris muris* in humans and mice, respectively. However, inappropriate activation of type 2 immune responses is also associated with detrimental conditions, such as asthma where smooth muscle constriction in the airways results in airway hyper-responsiveness (AHR). Although CD4$^+$ T helper type 2 (Th2) cells are a major player in type 2 immune responses because of their ability to express a wide variety of the classical effector cytokines, other cell types have also been shown to play important roles in the response. This review focuses on the mechanisms of generating type 2 immune responses, with particular focus on the antigens/allergens, cells and inflammatory mediators that are important for the initiation of these responses.

Abbreviations: AHR, airway hyper-responsiveness; CD, cluster of differentiation; DC, dendritic cell; GATA3, GATA-binding protein 3; HDM, house dust mite; HS2, DNase I-hypersensitivity site 2; IH2, innate helper 2; IL, interleukin; NBNT, non-B/non-T; NLR, NOD-like receptor; NLRP3, NLR family, pyrin domain containing 3; NOD, nucleotide oligomerisation domain; SEA, *Schistosoma mansoni* egg antigen; TCR, T-cell receptor; $T_{H,}$ T helper; TLR, Toll-like receptor; TSLP, thymic stromal lymphopoietin.

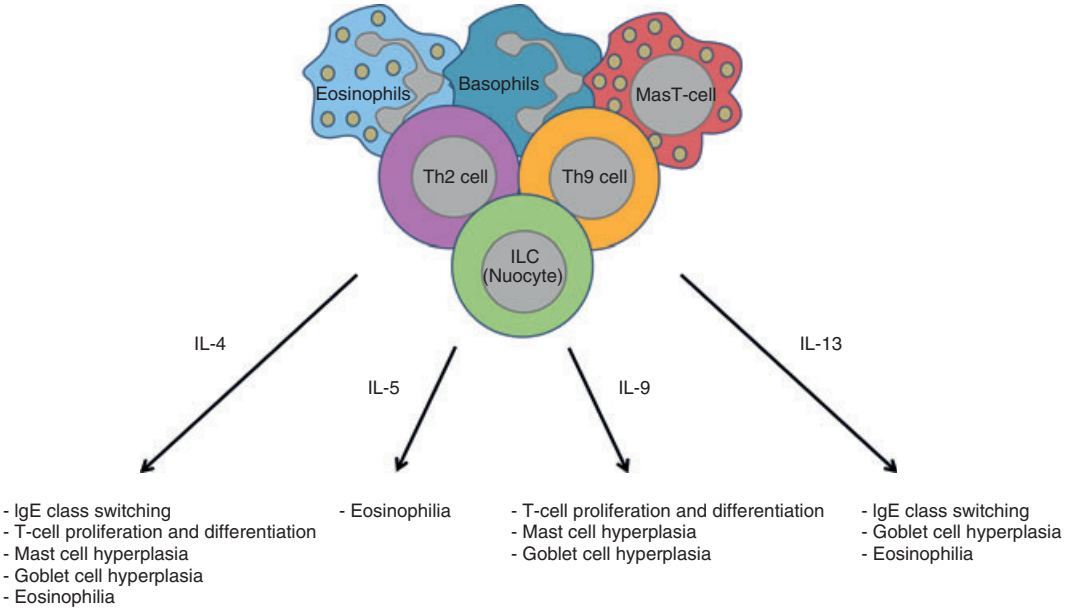

Figure 1. Expression and function of type 2 effector cytokines. Type 2 effector cytokines come from a variety of sources including antigen-stimulated $CD4^+$ T-cell subsets such as T helper type 2 (Th2) and Th9 cells, antigen-stimulated basophils or cytokine-stimulated innate lymphoid cells (ILC), such as nuocytes. It is the expression of these cytokines that leads to IgE class switching, goblet cell hyperplasia, which is important for mucus production, recruitment of various innate cell populations like eosinophils, basophils and mast cells, and enhanced the proliferation and differentiation of $CD4^+$ T cells. IL-4, interleukin-4.

What antigens are associated with type 2 immune responses?

To initiate an adaptive immune response, cells of the innate immune system must acquire antigens and process them into peptides that can be presented to $CD4^+$ T helper cells via MHC class II molecules. It is widely known that the antigens associated with Th1 and Th17 cell differentiation are acquired by phagocytosis and processing of the invading pathogen. However, the pathogens associated with type 2 immune responses are large and unlikely to be phagocytosed by antigen-presenting cells. Therefore, it is believed that proteins shed or excreted during the life cycle of the parasite are phagocytosed and presented to $CD4^+$ T cells via MHC class II.

Furthermore, efficient $CD4^+$ T-cell responses require additional signals from innate cells following the recognition of conserved molecular structures, known as microorganism-associated molecular patterns. Much of our knowledge regarding microorganism-associated molecular patterns and the guidance of the adaptive immune response comes from studies on Toll-like receptors (TLRs) and their role in guiding Th1 and Th17 responses. However, it is only recently that we have started to identify the receptors and antigens associated with the initiation of type 2 immune responses. For instance, there are increasing amounts of data demonstrating that cysteine proteases such as papain and house dust mite (HDM)-derived Der p 1 initiate type 2 immune responses *in vivo*.[1–4] Further to these findings *in vitro* studies have shown that epithelial cell lines and basophils treated with cysteine proteases express a variety of type 2 effector cytokines including IL-4 and thymic stromal lymphopoietin (TSLP).[4,5]

In addition to Der p 1, HDM allergen has also been shown to contain a second allergen known as Der p 2.[6] A study by Trompette *et al.*[7] demonstrated that Der p 2 is structurally similar to the TLR4-associated adapter protein MD2. A second study by Hammad *et al.*[8] used elegant chimera studies to show that HDM allergen administered to the airway only induced type 2 immune responses when TLR4 expression was present on non-haematopoietic cells. Furthermore, intranasal administration of Der p 2 in the absence of MD2 still induced airway inflammation.[7] In addition, several studies have shown that HDM preparations contain low levels of bacterial lipopolysaccharide, which is a known ligand for TLR4.[7] Interestingly, low concentrations of lipopolysaccharide administered to a host, combined with ovalbumin, induce a type 2 immune response associated with IgE class switching, eosinophil recruitment and expression of the effector cytokines IL-5 and IL-13.[9] Based on these findings it is possible to suggest that lung non-haematopoietic cells recognize low levels of lipopolysaccharide through a TLR4/Der p 2-dependent manner and that this may contribute to the initiation of type 2 immune responses (Fig. 2).

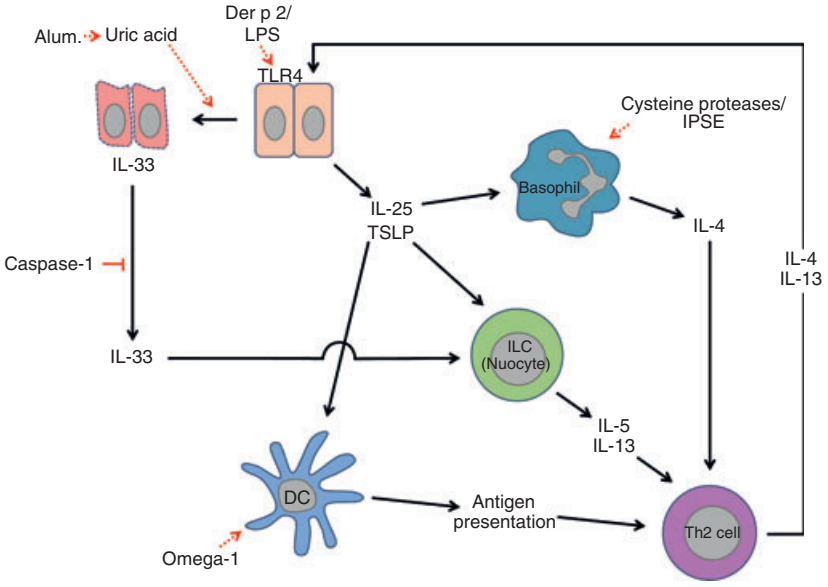

Figure 2. A complex cytokine network underlies the initiation of type 2 immune responses. Antigen stimulation, leads to thymic stromal lymphopoietin (TSLP) or interleukin-25 (IL-25) expression from non-haematopoietic cell sources that subsequently induce type 2 effector cytokine expression from various cell populations including innate lymphoid cells, basophils or $CD4^+$ T cells. Alternatively, IL-33, which induces many of the features similar to IL-25 and TSLP, is released as an active form from necrotic cells and acts as an alarmin. In addition, certain antigens such as Omega-1 from *Schistosoma mansoni* condition dendritic cells (DCs) so that they guide $CD4^+$ T cells towards a type 2 response. IPSE, inducing principle of *Schistosoma mansoni*; Th2, T helper type 2; TLR4, Toll-like receptor 4.

It has long been known that type 2 immune responses can be initiated against antigens, such as ovalbumin, by combining them with aluminium-containing adjuvants, collectively known as 'alum'. Although alum is extensively used as an adjuvant in humans its precise mechanism of action is poorly understood. One of the major insights into the mechanism of alum came from the observation that *in vitro* stimulated dendritic cells (DCs) expressed functional IL-1β, IL-18 and, particularly important for type 2 immune responses, IL-33.[10,11] A later study by the same group highlighted an important role for caspase-1 in alum-induced expression of IL-1 family members.[10] Further *in vitro* studies identified that alum-mediated caspase-1 activation was dependent on the NOD-like receptor (NLR) family, pyrin domain containing (NLRP3) inflammasome and its appropriate adaptor molecules.[12–16] The importance of the NLRP3 inflammasome in alum-induced type 2 immune responses was highlighted *in vivo* by findings that mice deficient in NLRP3 had impaired class switching to IgE following alum administration.[12,13] Based on these findings it was thought that activating the NLRP3 inflammasome resulted in caspase-1 activation and the subsequent activation of IL-33. In contrast to this hypothesis, recent studies have suggested that biologically active IL-33 is constitutively expressed by epithelial/endothelial cells and inactivated by caspases.[17–19] Furthermore, a splice variant of IL-33 lacking the caspase-1 cleavage site is constitutively active.[20] The studies described above have shed light on the mechanism behind alum-induced type 2 immune responses although the findings are still controversial. It is possible that alum causes tissue damage, potentially through a build up of uric acid,[21] and subsequently the release of biologically active IL-33 plays an important role in the expression of type 2 cytokines from various cell types (Fig. 2).[11]

In addition to specific antigens or allergens, *in vitro* studies have shown that Th2 differentiation, which could lead to type 2 immune responses, is also dependent on the quality of the T-cell receptor signal. For instance, early studies showed that activating $CD4^+$ T cells with low and high doses of antigen induced a Th2 and Th1 differentiation, respectively.[22,23] Similarly stimulation of naive $CD4^+$ T cells with altered peptides is associated with increased Th2 differentiation.[24,25] Weak T-cell receptor stimulation functions by up-regulating the expression of GATA-binding protein 3 (GATA3), which is an essential step in the differentiation of Th2 cells, leading to IL-4 expression. Interestingly, a collection of studies hypothesized that a major antigen in *S. mansoni* soluble egg antigen (SEA) known as omega-1 conditions DCs resulting in a decrease in T-cell receptor signalling strength.[26,27]

It is important to note that SEA preparations deficient in omega-1 are still able to induce Th2 polarization.[26] The ability of SEA to induce Th2 differentiation can be explained by the presence of glycosylated components found in the preparation that can act as adjuvants. For instance, a study by Schramm *et al.*[28] showed that the SEA component known as inducing principle of

S. mansoni eggs induced IL-4 expression from basophils through an IgE-dependent mechanism. Furthermore, intranasal immunizations with human serum albumin conjugated to lacto-*N*-fucopentose III resulted in the initiation of a type 2 immune responses.[29]

Overall these studies have increased our understanding of the antigens and allergens involved in the initiation of type 2 immune responses. However, they clearly highlight that initiation of type 2 immune responses is dependent on a variety of mediators expressed by a wide range of haematopoietic and non-haematopoietic cell types.

Which cytokines are important for the initiation of type 2 immune responses?

Many of the antigens and allergens described above do not act directly on $CD4^+$ T cells and therefore initiate Th2 differentiation through intermediate mediators. It has long been known that IL-4 is a characteristic type 2 cytokine, and based on early *in vitro* studies it was thought to play an essential role in the differentiation of Th2 cells.[30,31] However, since these studies, *in vivo* analysis has shown that Th2 cell differentiation can occur in the absence of IL-4 signalling.[32,33] Therefore, suggesting that IL-4 signalling influences the number of Th2 cells that develop *in vivo* but is not essential. It is now widely accepted that IL-4 signalling leads to GATA3 up-regulation and an indispensable role in the expression of classical effector cytokines such as IL-5 and IL-13.[34,35] Furthering our understanding of this field, a recent study by Tanaka *et al.*[36] demonstrated that the expression of IL-4 by Th2 cells required GATA3 binding to a *cis*-acting regulatory element, which is known as DNase I-hypersensitive site 2 (HS2), in the second intron of the IL-4 locus. Interestingly, T cells deficient in HS2 showed impaired expression of IL-4, whereas other type 2 cytokines including IL-13 and IL-5 were unaffected.[36] In addition to IL-4/GATA3-dependent mechanisms, it is now widely accepted that cytokines, such as TSLP, IL-25 (also known as IL-17E) and IL-33, from non-lymphoid sources are important for Th2 cell differentiation.

Thymic stromal lymphopoietin

Since its discovery, TSLP expression has been observed from epithelial cells, basophils and mast cells.[4,28,37–39] The importance of TSLP in the initiation of type 2 immune responses is highlighted by the observation that specific over-expression of this cytokine in the lung results in airway inflammation characterized by increased AHR.[40] As confirmation of these results, TSLPR-deficient mice had impaired type 2 responses in an ovalbumin-induced asthma model.[40] Similar results have been seen in mice treated with a soluble TSLPR-immunoglobulin fusion protein.[41] Confirming the importance of TSLP in type 2 immune responses recent *in vivo* studies have shown that TSLP signalling is important for the clearance of *T. muris*.[38,42] However, the role of TSLP in the initiation of type 2 immune responses during other helminth infections is not so clear, because TSLPR-deficient mice generate normal type 2 immune responses and can clear *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus* infections similar to wild-type controls.[42] It is believed that TSLP functions by inhibiting the expression of type 1 polarizing cytokines, such as interferon-γ and IL-12p70, because depleting antibodies against these cytokines in TSLPR-deficient mice rescues their ability to generate type 2 immune responses and clear the parasite.[38,42] Therefore, it is likely that parasites, such as *T. muris*, that induce a mixture of type 1 and type 2 cytokines require TSLP to inhibit the expression of the former.

In addition, TSLP has been shown to condition DCs by enhancing their expression of chemokines capable of attracting Th2 cells expressing CCR4,[37] up-regulating OX40 ligand expression, which is important for GATA3 expression *in vitro*,[43] and activating mast cells.[44] Although a large body of evidence suggests that TSLP functions by conditioning DCs and other innate cells, it has also been shown that the allergic response to inhaled antigens is rescued in TSLPR-deficient mice by transferring wild-type $CD4^+$ T cells.[45] There is some suggestion that TSLP acts directly on $CD4^+$ T cells by inducing signal transducer and activator of transcription-5 phosphorylation, a process that is important for the expression of IL-4.[46,47]

Interleukin-25

Interleukin-25, also known as IL-17E, was discovered using cDNA libraries from Th2-polarized cells.[48] Other sources of IL-25 include epithelial cells, basophils, eosinophils and mast cells.[49–53] The importance of IL-25 in the initiation of type 2 immune responses was highlighted in studies where intraperitoneal injection of recombinant IL-25 induced features associated with type 2 immune responses.[48] Furthermore, IL-25 is detrimental in allergic lung diseases, as over-expression of IL-25 in airway epithelial cells results in the initiation of type 2 responses.[49] Similarly, intranasal administration of IL-25 leads to increased AHR, an effect that can be inhibited by antibodies against IL-25 or its receptor.[54] Furthermore, two independent studies showed that airway inflammation and AHR were reduced in *IL-25*$^{-/-}$ mice or BALB/c mice treated with an IL-25 neutralizing antibody during an ovalbumin-induced asthma model.[55,56] In humans, polymorphisms in *IL-17RB*, which pairs with *IL-17RA* to form the IL-25 receptor, have been associated with asthma.[57]

In contrast, IL-25 has been shown to be beneficial in various helminth infections. For instance, animals deficient in IL-25 signalling show delayed or absent expulsion

of the intestinal helminths *N. brasiliensis* or *T. muris*, respectively,[58–60] whereas IL-25 administration rapidly enhances worm expulsion in *N. brasiliensis* infections.[58] Interestingly studies with *N. brasiliensis*-infected *Rag2*$^{-/-}$ mice showed that IL-25-dependent induction of IL-4 required T and B lymphocytes, but IL-5 and IL-13 were expressed by non-B/non-T (NBNT) cells.[48,60,61]

Interleukin-33

Interleukin-33 is a member of the IL-1 family[11] and signals through T1/ST2 (encoded by *Il1rl1*) a receptor expressed by murine Th2 cells.[62,63] The importance of IL-33 in the initiation of type 2 immune responses is highlighted by the observation that administration of this cytokine resulted in eosinophilia, IgE class switching, production of classical type 2 cytokines, and changes in mucus production in the lung and gastrointestinal tract.[11] Further confirming the importance of IL-33 is the observation that AHR development is impaired in mice deficient in this cytokine compared with wild-type controls[64] and blocking T1/ST2 with an antibody or fusion protein decreased many of the characteristics associated with type 2 responses following the adoptive transfer of Th2 cells.[63]

Similar to IL-25, IL-33 also plays beneficial roles as *Il1rl1*$^{-/-}$ mice are impaired in their ability to express type 2 cytokines following *S. mansoni* egg administration and consequently these animals have impaired granuloma formation.[65] Furthermore, administration of exogenous IL-33 guides the immune response towards a beneficial type 2 response during *T. muris* infections.[66] Although *N. brasiliensis* expulsion is normal in *Il1rl1*$^{-/-}$ mice, suggesting that IL-33 signalling plays a minor role, the expulsion of this parasite was dramatically impaired in *Il17br*$^{-/-}$ *Il1rl1*$^{-/-}$ mice compared with mice deficient in *Il17br* alone.[60]

As mentioned earlier there is an increasing body of evidence suggesting that IL-33 is not secreted but instead acts as an alarmin. For instance, IL-33 is constitutively expressed in the nucleus of human epithelial and endothelial cells and released following damage or injury.[17] Furthermore, unlike other IL-1 family members, IL-33 is not activated by caspase-1, in fact there is evidence to suggest that caspase-1 inactivates IL-33.[18–20] This suggests that apoptotic cells inactivate IL-33 thereby preventing the development of type 2 immune responses, whereas necrotic cells, that do not activate caspase-1, release biologically active IL-33 and initiate type 2 immune responses.

Interleukin-33 appears to regulate the type 2 response through a number of pathways including the induction of IL-13 and IL-5 expression from NBNT cell sources.[60,61] Furthermore, IL-33 has been associated with increased IL-4 and IL-13 expression by human basophils and enhanced histamine release when combined with IgE cross-linking.[67,68] Interleukin-33 has also been shown to help initiate type 2 immune responses by activating human mast cells and inducing the differentiation of alternatively activated macrophages.[69]

Innate cell responses associated with type 2 immune responses

During type 2 immune responses, T cells are a major source of cytokines. However the observation that T-cell-deficient and B-cell-deficient *Rag2*$^{-/-}$ mice are still able to express IL-5 and IL-13 following IL-25 treatment suggested an NBNT cell source of these cytokines.[48] A later study found that IL-25 was important for the expansion of NBNT cells during *N. brasiliensis* infection and that the absence of this population correlated with an inability to expel the parasite.[58] As these original studies several groups have identified and characterized NBNT cell populations that are important for type 2 immune responses.

Innate lymphoid cells

One of the first studies came from Neill *et al.*,[60] who followed up on their original identification of an NBNT cell in *N. brasiliensis* infection by using an IL-13–enhanced green fluorescent protein reporter mouse to identify cellular sources of this cytokine following *N. brasiliensis* infection or the administration of exogenous IL-25 or IL-33. Using this approach, Neill *et al.*[60] identified that a major source of IL-13 came from a previously undescribed cell that did not express any known lineage markers. Based on their expression of IL-13, these cells were termed nuocytes after the 13th letter of the Greek alphabet.[60] Demonstrating an important role for nuocytes in helminth infections, Neill *et al.*[60] showed that the adoptive transfer of these cells into *Il17br*$^{-/-}$ *Il1rl1*$^{-/-}$ mice, which have impaired type 2 immune responses and are unable to expel *N. brasiliensis*, rescued their ability to expel the parasite. Furthermore, through elegant transfer experiments with IL-13-deficient nuocytes this study demonstrated that IL-13 from nuocytes alone was sufficient to induce parasite expulsion.[60]

A separate study by Moro *et al.*[61] identified an innate lymphoid cell in distinct lymphoid structures, which were termed fat-associated lymphoid structures, in the adipose tissues of the peritoneal cavity. This cell was characterized by its expression of c-kit and the absence of any known lineage markers, a similar surface phenotype to the NBNT cells described by Fallon *et al.* and Moro *et al.*[58,61] termed them natural helper cells and showed that they expressed various type 2 cytokines including IL-5 and IL-13. Similarly, a separate study by Price *et al.*[70] identified an IL-13 and IL-5 innate cell population, which were termed innate helper 2 (IH2) cells, in the spleen, mesenteric lymph nodes, peritoneum and liver of IL-25-treated hosts. Both Price *et al.* and Moro *et al.*[61,70] showed that these innate

cell populations were absent from $\gamma c^{-/-}$ *Rag2*$^{-/-}$ mice, and that this correlated with an inability to expel *N. brasiliensis*. Similar to Neill *et al.*, Moro *et al.*[60,61] demonstrated that adoptively transferred natural helper cells rescued goblet cell hyperplasia, which is important for helminth expulsion, in *N. brasiliensis*-infected $\gamma c^{-/-}$ *Rag2*$^{-/-}$ mice, and were shown to be important for the proliferation of B1 cells potentially through the expression of IL-5. In contrast, Price *et al.*[70] showed that adoptively transferred IH2 cells alone were not sufficient to induce *N. brasiliensis* expulsion from $\gamma c^{-/-}$ *Rag2*$^{-/-}$ mice, instead worm expulsion required both IH2 cells and exogenous IL-25 administration. Similarly, eosinophilia in the lung and spleen required both adoptively transferred IH2 cells and exogenous IL-25 administration.[70] Together these studies clearly demonstrate an important role for these innate lymphoid populations in the development of type 2 immune responses required for parasite expulsion. Further investigation is required to determine how the cells described in these studies relate to each other.

Multipotent progenitor cells

Around the same time as the studies described above, a fourth study from Saenz *et al.*[71] used an IL-4–enhanced green fluorescent protein reporter mouse to show that IL-25 administration amplified a population of NBNT c-Kit$^+$ IL-4$^+$ cells and a population of NBNT c-Kit$^+$ IL-4$^-$ cells. This study demonstrated a role for NBNT c-Kit$^+$ IL-4$^-$ cells, which were termed multipotent progenitor (MPP)^{type2} cells, in type 2 immunity because adoptive transfer of these cells rescued the ability to generate type 2 responses in *T. muris*-infected *IL-25*$^{-/-}$ mice. Although MPP^{type2} cells are clearly important for type 2 immunity the study by Saenz *et al.*[71] did not report expression of IL-13 or IL-5 like the innate lymphoid populations described previously.[60,61,70] Furthermore, based on their ability to differentiate into various innate cell populations including basophils, mast cells and macrophages it appears that MPP^{type2} cells are a distinct or mixed cell population.[71]

Basophils

The identification of these new innate lymphoid cells has added to the complexity of the initiation of type 2 immune responses, which had focused on the role of the basophil. In recent years we have started to understand the effector functions of basophils. Previously it has been described that murine and human basophils are an early source of IL-4,[4,28,72–74] which as discussed earlier influences Th2 differentiation.[32,33] The potential role of basophils as antigen-presenting cells was recently suggested by several studies,[4,75,76] however, Ohnmacht *et al.*[77] using *Mcpt8Cre* mice, which are deficient in basophils, has recently shown that these cells are not important for papain-induced Th2 cell differentiation or protection against primary infections with *N. brasiliensis*. However, basophils were found to be important for protection against secondary *N. brasiliensis* infections and the onset of chronic allergic dermatitis.[77] A further study by Sullivan *et al.*[78] clearly refutes the idea of basophils as antigen-presenting cells because they showed that Th2 cytokine responses were normal in basophil-deficient Basoph8 × Rosa-DTα mice injected with *S. mansoni* eggs. From here it will be important for future studies to use mice deficient in this cell type such as that described by Ohnmacht *et al.* or the *Mcpt8*-diphtheria toxin receptor inducible system to elucidate the function of this population.[77–79] Since the early investigations, a study by Hammad *et al.*[80] identified an inflammatory DC population recruited to the lung draining lymph node of HDM-treated hosts. This recruited CD11c$^+$ MHC class II$^+$ population was found to express FcεR1 and would therefore be depleted by the MAR1 antibody used previously.[4,80,81] Furthermore, *in vitro* co-cultures with FcεR1$^+$ DCs or basophils showed that the former were important for the expression of type 2 effector cytokines by CD4$^+$ T cells.[80]

Conclusion

In recent years our knowledge of the initiating factors of type 2 immune responses has increased dramatically, however because of the extensive redundancy within the system there is still much to learn. The essential roles of these responses in both helminth infections, which affect the health of many individuals in developing countries, and inflammatory conditions such as asthma that affects over 300 million people worldwide, highlights the need to understand these complex responses. It is to be hoped that such elucidation will lead to the identification of new therapeutic targets in these pathways.

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Disclosures

The authors have no conflicts of interest.

References

1 McKerrow JH, Caffrey C, Kelly B, Loke P, Sajid M. Proteases in parasitic diseases. *Annu Rev Pathol* 2006; **1**:497–536.

2 Gough L, Schulz O, Sewell HF, Shakib F. The cysteine protease activity of the major dust mite allergen *Der p* 1 selectively enhances the immunoglobulin E antibody response. *J Exp Med* 1999; **190**:1897–902.

3 Chambers L, Brown A, Pritchard DI, Sreedharan S, Brocklehurst K, Kalsheker NA. Enzymatically active papain preferentially induces an allergic response in mice. *Biochem Biophys Res Commun* 1998; **253**:837–40.

4 Sokol CL, Barton GM, Farr AG, Medzhitov R. A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nat Immunol* 2008; **9**:310–8.

5 Kouzaki H, O'Grady SM, Lawrence CB, Kita H. Proteases induce production of thymic stromal lymphopoietin by airway epithelial cells through protease-activated receptor-2. *J Immunol* 2009; **183**:1427–34.

6 Park GM, Lee SM, Lee IY, Ree HI, Kim KS, Hong CS, Yong TS. Localization of a major allergen, *Der p* 2, in the gut and faecal pellets of *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 2000; **30**:1293–7.

7 Trompette A, Divanovic S, Visintin A *et al.* Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. *Nature* 2009; **457**:585–8.

8 Hammad H, Chieppa M, Perros F, Willart MA, Germain RN, Lambrecht BN. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat Med* 2009; **15**:410–6.

9 Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 2002; **196**:1645–51.

10 Li H, Nookala S, Re F. Aluminum hydroxide adjuvants activate caspase-1 and induce IL-1beta and IL-18 release. *J Immunol* 2007; **178**:5271–6.

11 Schmitz J, Owyang A, Oldham E *et al.* IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005; **23**:479–90.

12 Li H, Willingham SB, Ting JP, Re F. Cutting edge: inflammasome activation by alum and alum's adjuvant effect are mediated by NLRP3. *J Immunol* 2008; **181**:17–21.

13 Kool M, Petrilli V, De Smedt T *et al.* Cutting edge: alum adjuvant stimulates inflammatory dendritic cells through activation of the NALP3 inflammasome. *J Immunol* 2008; **181**:3755–9.

14 Hornung V, Bauernfeind F, Halle A, Samstad EO, Kono H, Rock KL, Fitzgerald KA, Latz E. Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nat Immunol* 2008; **9**:847–56.

15 Eisenbarth SC, Colegio OR, O'Connor W, Sutterwala FS, Flavell RA. Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. *Nature* 2008; **453**:1122–6.

16 Franchi L, Nunez G. The Nlrp3 inflammasome is critical for aluminium hydroxide-mediated IL-1beta secretion but dispensable for adjuvant activity. *Eur J Immunol* 2008; **38**:2085–9.

17 Moussion C, Ortega N, Girard JP. The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells *in vivo*: a novel 'alarmin'? *PLoS One* 2008; **3**:e3331.

18 Cayrol C, Girard JP. The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. *Proc Natl Acad Sci U S A* 2009; **106**:9021–6.

19 Talabot-Ayer D, Lamacchia C, Gabay C, Palmer G. Interleukin-33 is biologically active independently of caspase-1 cleavage. *J Biol Chem* 2009; **284**:19420–6.

20 Hong J, Bae S, Jhun H *et al.* Identification of constitutively active IL-33 splice variant. *J Biol Chem* 2011; **286**:20078–86.

21 Kool M, Soullie T, van Nimwegen M *et al.* Alum adjuvant boosts adaptive immunity by inducing uric acid and activating inflammatory dendritic cells. *J Exp Med* 2008; **205**:869–82.

22 Hosken NA, Shibuya K, Heath AW, Murphy KM, O'Garra A. The effect of antigen dose on $CD4^+$ T helper cell phenotype development in a T cell receptor-alpha beta-transgenic model. *J Exp Med* 1995; **182**:1579–84.

23 Constant S, Pfeiffer C, Woodard A, Pasqualini T, Bottomly K. Extent of T cell receptor ligation can determine the functional differentiation of naive $CD4^+$ T cells. *J Exp Med* 1995; **182**:1591–6.

24 Pfeiffer C, Stein J, Southwood S, Ketelaar H, Sette A, Bottomly K. Altered peptide ligands can control CD4 T lymphocyte differentiation *in vivo*. *J Exp Med* 1995; **181**:1569–74.

25 Tao X, Grant C, Constant S, Bottomly K. Induction of IL-4-producing $CD4^+$ T cells by antigenic peptides altered for TCR binding. *J Immunol* 1997; **158**:4237–44.

26 Everts B, Perona-Wright G, Smits HH *et al.* Omega-1, a glycoprotein secreted by *Schistosoma mansoni* eggs, drives Th2 responses. *J Exp Med* 2009; **206**:1673–80.

27 Steinfelder S, Andersen JF, Cannons JL *et al.* The major component in schistosome eggs responsible for conditioning dendritic cells for Th2 polarization is a T2 ribonuclease (omega-1). *J Exp Med* 2009; **206**:1681–90.

28 Schramm G, Mohrs K, Wodrich M, Doenhoff MJ, Pearce EJ, Haas H, Mohrs M. Cutting edge: IPSE/alpha-1, a glycoprotein from *Schistosoma mansoni* eggs, induces IgE-dependent, antigen-independent IL-4 production by murine basophils *in vivo*. *J Immunol* 2007; **178**:6023–7.

29 Okano M, Satoskar AR, Nishizaki K, Harn DA Jr. Lacto-*N*-fucopentaose III found on *Schistosoma mansoni* egg antigens functions as adjuvant for proteins by inducing Th2-type response. *J Immunol* 2001; **167**:442–50.

30 Le Gros G, Ben-Sasson SZ, Seder R, Finkelman FD, Paul WE. Generation of interleukin 4 (IL-4)-producing cells *in vivo* and *in vitro*: IL-2 and IL-4 are required for *in vitro* generation of IL-4-producing cells. *J Exp Med* 1990; **172**:921–9.

31 Swain SL, Weinberg AD, English M, Huston G. IL-4 directs the development of Th2-like helper effectors. *J Immunol* 1990; **145**:3796–806.

32 Jankovic D, Kullberg MC, Noben-Trauth N, Caspar P, Paul WE, Sher A. Single cell analysis reveals that IL-4 receptor/Stat6 signaling is not required for the *in vivo* or *in vitro* development of $CD4^+$ lymphocytes with a Th2 cytokine profile. *J Immunol* 2000; **164**:3047–55.

33 Finkelman FD, Morris SC, Orekhova T *et al.* Stat6 regulation of *in vivo* IL-4 responses. *J Immunol* 2000; **164**:2303–10.

34 Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* 1997; **89**:587–96.

35 Zhu J, Min B, Hu-Li J *et al.* Conditional deletion of Gata3 shows its essential function in T_H1-T_H2 responses. *Nat Immunol* 2004; **5**:1157–65.

36 Tanaka S, Motomura Y, Suzuki Y, Yagi R, Inoue H, Miyatake S, Kubo M. The enhancer HS2 critically regulates GATA-3-mediated *Il4* transcription in T_H2 cells. *Nat Immunol* 2011; **12**:77–85.

37 Soumelis V, Reche PA, Kanzler H *et al.* Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002; **3**:673–80.

38 Taylor BC, Zaph C, Troy AE, Du Y, Guild KJ, Comeau MR, Artis D. TSLP regulates intestinal immunity and inflammation in mouse models of helminth infection and colitis. *J Exp Med* 2009; **206**:655–67.

39 Friend SL, Hosier S, Nelson A, Foxworthe D, Williams DE, Farr A. A thymic stromal cell line supports *in vitro* development of surface IgM^+ B cells and produces a novel growth factor affecting B and T lineage cells. *Exp Hematol* 1994; **22**:321–8.

40 Zhou B, Comeau MR, De Smedt T *et al.* Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat Immunol* 2005; **6**:1047–53.

41 Zhang F, Huang G, Hu B, Song Y, Shi Y. A soluble thymic stromal lymphopoietin (TSLP) antagonist, TSLPR-immunoglobulin, reduces the severity of allergic disease by regulating pulmonary dendritic cells. *Clin Exp Immunol* 2011; **164**:256–64.

42 Massacand JC, Stettler RC, Meier R, Humphreys NE, Grencis RK, Marsland BJ, Harris NL. Helminth products bypass the need for TSLP in Th2 immune responses by directly modulating dendritic cell function. *Proc Natl Acad Sci U S A* 2009; **106**:13968–73.

43 Ito T, Wang YH, Duramad O *et al.* TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med* 2005; **202**:1213–23.

44 Allakhverdi Z, Comeau MR, Jessup HK *et al.* Thymic stromal lymphopoietin is released by human epithelial cells in response to microbes, trauma, or inflammation and potently activates mast cells. *J Exp Med* 2007; **204**:253–8.

45 Al-Shami A, Spolski R, Kelly J, Keane-Myers A, Leonard WJ. A role for TSLP in the development of inflammation in an asthma model. *J Exp Med* 2005; **202**:829–39.

46 Isaksen DE, Baumann H, Trobridge PA, Farr AG, Levin SD, Ziegler SF. Requirement for STAT5 in thymic stromal lymphopoietin-mediated signal transduction. *J Immunol* 1999; **163**:5971–7.

47 Al-Shami A, Spolski R, Kelly J, Fry T, Schwartzberg PL, Pandey A, Mackall CL, Leonard WJ. A role for thymic stromal lymphopoietin in $CD4^+$ T cell development. *J Exp Med* 2004; **200**:159–68.

48 Fort MM, Cheung J, Yen D *et al.* IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies *in vivo*. *Immunity* 2001; **15**:985–95.

49 Angkasekwinai P, Park H, Wang YH, Chang SH, Corry DB, Liu YJ, Zhu Z, Dong C. Interleukin 25 promotes the initiation of proallergic type 2 responses. *J Exp Med* 2007; **204**:1509–17.

50 Zhao A, Urban JF Jr, Sun R *et al.* Critical role of IL-25 in nematode infection-induced alterations in intestinal function. *J Immunol* 2010; **185**:6921–9.

51 Wang YH, Angkasekwinai P, Lu N *et al.* IL-25 augments type 2 immune responses by enhancing the expansion and functions of TSLP-DC-activated Th2 memory cells. *J Exp Med* 2007; **204**:1837–47.

52 Terrier B, Bieche I, Maisonobe T *et al.* Interleukin-25: a cytokine linking eosinophils and adaptive immunity in Churg–Strauss syndrome. *Blood* 2010; **116**:4523–31.

53 Dolgachev V, Petersen BC, Budelsky AL, Berlin AA, Lukacs NW. Pulmonary IL-17E (IL-25) production and $IL\text{-}17RB^+$ myeloid cell-derived Th2 cytokine production are dependent upon stem cell factor-induced responses during chronic allergic pulmonary disease. *J Immunol* 2009; **183**:5705–15.

54 Rickel EA, Siegel LA, Yoon BR *et al.* Identification of functional roles for both IL-17RB and IL-17RA in mediating IL-25-induced activities. *J Immunol* 2008; **181**:4299–310.

55 Ballantyne SJ, Barlow JL, Jolin HE *et al.* Blocking IL-25 prevents airway hyperresponsiveness in allergic asthma. *J Allergy Clin Immunol* 2007; **120**:1324–31.

56 Angkasekwinai P, Chang SH, Thapa M, Watarai H, Dong C. Regulation of IL-9 expression by IL-25 signaling. *Nat Immunol* 2010; **11**:250–6.

57 Jung JS, Park BL, Cheong HS *et al.* Association of IL-17RB gene polymorphism with asthma. *Chest* 2009; **135**:1173–80.

58 Fallon PG, Ballantyne SJ, Mangan NE *et al.* Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. *J Exp Med* 2006; **203**:1105–16.

59 Owyang AM, Zaph C, Wilson EH *et al.* Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammation in the gastrointestinal tract. *J Exp Med* 2006; **203**:843–9.

60 Neill DR, Wong SH, Bellosi A *et al.* Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature* 2010; **464**:1367–70.

61 Moro K, Yamada T, Tanabe M *et al.* Innate production of T_H2 cytokines by adipose tissue-associated c-Kit$^+$ Sca-1$^+$ lymphoid cells. *Nature* 2010; **463**:540–4.

62 Xu D, Chan WL, Leung BP, Huang F, Wheeler R, Piedrafita D, Robinson JH, Liew FY. Selective expression of a stable cell surface molecule on type 2 but not type 1 helper T cells. *J Exp Med* 1998; **187**:787–94.

63 Lohning M, Stroehmann A, Coyle AJ *et al.* T1/ST2 is preferentially expressed on murine Th2 cells, independent of interleukin 4, interleukin 5, and interleukin 10, and important for Th2 effector function. *Proc Natl Acad Sci U S A* 1998; **95**:6930–5.

64 Oboki K, Ohno T, Kajiwara N *et al.* IL-33 is a crucial amplifier of innate rather than acquired immunity. *Proc Natl Acad Sci U S A* 2010; **107**:18581–6.

65 Townsend MJ, Fallon PG, Matthews DJ, Jolin HE, McKenzie AN. T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses. *J Exp Med* 2000; **191**:1069–76.

66 Humphreys NE, Xu D, Hepworth MR, Liew FY, Grencis RK. IL-33, a potent inducer of adaptive immunity to intestinal nematodes. *J Immunol* 2008; **180**:2443–9.

67 Suzukawa M, Iikura M, Koketsu R *et al.* An IL-1 cytokine member, IL-33, induces human basophil activation via its ST2 receptor. *J Immunol* 2008; **181**:5981–9.

68 Pecaric-Petkovic T, Didichenko SA, Kaempfer S, Spiegl N, Dahinden CA. Human basophils and eosinophils are the direct target leukocytes of the novel IL-1 family member IL-33. *Blood* 2009; **113**:1526–34.

69 Kurowska-Stolarska M, Stolarski B, Kewin P *et al.* IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. *J Immunol* 2009; **183**:6469–77.

70 Price AE, Liang HE, Sullivan BM, Reinhardt RL, Eisley CJ, Erle DJ, Locksley RM. Systemically dispersed innate IL-13-expressing cells in type 2 immunity. *Proc Natl Acad Sci U S A* 2010; **107**:11489–94.

71 Saenz SA, Siracusa MC, Perrigoue JG *et al.* IL25 elicits a multipotent progenitor cell population that promotes T_H2 cytokine responses. *Nature* 2010; **464**:1362–6.

72 Voehringer D, Shinkai K, Locksley RM. Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. *Immunity* 2004; **20**:267–77.

73 Min B, Prout M, Hu-Li J *et al.* Basophils produce IL-4 and accumulate in tissues after infection with a Th2-inducing parasite. *J Exp Med* 2004; **200**:507–17.

74 Mitre E, Taylor RT, Kubofcik J, Nutman TB. Parasite antigen-driven basophils are a major source of IL-4 in human filarial infections. *J Immunol* 2004; **172**:2439–45.

75 Perrigoue JG, Saenz SA, Siracusa MC *et al.* MHC class II-dependent basophil-CD4$^+$ T cell interactions promote T_H2 cytokine-dependent immunity. *Nat Immunol* 2009; **10**:697–705.

76 Yoshimoto T, Yasuda K, Tanaka H, Nakahira M, Imai Y, Fujimori Y, Nakanishi K. Basophils contribute to T_H2-IgE responses *in vivo* via IL-4 production and presentation of peptide–MHC class II complexes to CD4$^+$ T cells. *Nat Immunol* 2009; **10**:706–12.

77 Ohnmacht C, Schwartz C, Panzer M, Schiedewitz I, Naumann R, Voehringer D. Basophils orchestrate chronic allergic dermatitis and protective immunity against helminths. *Immunity* 2010; **33**:364–74.

78 Sullivan BM, Liang HE, Bando JK, Wu D, Cheng LE, McKerrow JK, Allen CD, Locksley RM. Genetic analysis of basophil function *in vivo*. *Nat Immunol* 2011; **12**:527–35.

79 Wada T, Ishiwata K, Koseki H *et al.* Selective ablation of basophils in mice reveals their nonredundant role in acquired immunity against ticks. *J Clin Invest* 2010; **120**:2867–75.

80 Hammad H, Plantinga M, Deswarte K, Pouliot P, Willart MA, Kool M, Muskens F, Lambrecht BN. Inflammatory dendritic cells – not basophils – are necessary and sufficient for induction of Th2 immunity to inhaled house dust mite allergen. *J Exp Med* 2010; **207**:2097–111.

81 Sokol CL, Chu NQ, Yu S, Nish SA, Laufer TM, Medzhitov R. Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nat Immunol* 2009; **10**:713–20.